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Characterization of two isoforms of cbb_3 cytochrome oxidase from *Pseudomonas stutzeri* ZoBell

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The bacterial cbb_3 cytochrome oxidase is a member of the heme-copper oxidase (HCO) superfamily, which couples the reduction of molecular oxygen to transmembrane proton pumping. The cbb_3 oxidases are characterized by a higher catalytic activity at low oxygen concentrations [1]. The presence of the cbb_3 cytochrome oxidase is considered to be essential for the pathogenicity of many bacterial species.

Pseudomonas stutzeri ZoBell contains two independent operons encoding cbb_3-isoforms, Cbb_3-1 and Cbb_3-2, respectively [2]. The X-ray structure of Cbb_3-1 at 3.2 Å resolution showed an electron transfer pathway that differs from the mitochondria-like oxidases (family A) and the *Thermus thermophilus* ba_3-like oxidase (family B). Moreover, structural differences around heme *b* and heme b_3-Cu_B binuclear center and the existence of one proton pathway possibly indicate a different redox-driven proton pumping mechanism.

To investigate structural and functional details of both isoforms, each of the two operons has been deleted by homologous recombination. These deletion strains are used for the expression of recombinant tagged cbb_3-isoforms. Both homologously produced isoforms were isolated and identified by in-gel detection using TMBZ/H_2O_2 assay and electrospray ionization mass spectrometry (ESI-MS). Compared with Cbb_3-1, Cbb_3-2 showed a low level of expression under both aerobic and micro-aerobic growth conditions. Promoter exchange has been used to increase the yield of Cbb_3-2. Both purified isoforms showed similar oxygen reduction activities. No significant difference was found between the wild-type cbb_3 oxidase and both recombinant isoforms.

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Non-invasive modulation of cytochrome *c* oxidase activity with specific infrared light for the treatment of reperfusion injury

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Reperfusion injury plays a major role in tissue damage in many diseases including stroke and myocardial infarction, when oxygen is let back into the ischemic tissue. The pathologic effect of reperfusion has largely been attributed to the production of reactive oxygen species (ROS) by mitochondria, which are generated at high mitochondrial membrane potentials ($\Delta\Psi_m$). Traditional pharmacologic therapies attempting to scavenge ROS have failed, possibly due to inherent difficulties in delivery of drugs to sub-cellular targets during the crucial early reperfusion phase. Here, we applied a non-pharmacologic strategy, that targets cytochrome *c* oxidase (COX), which acts as a photo-acceptor for infrared light (IRL). We discovered 4 specific IRL wavelengths that reduce the activity of COX. Our model proposes that such IRL should normalize $\Delta\Psi_m$ through inhibition of COX, which contributes to the generation of $\Delta\Psi_m$. We tested this hypothesis in an adult rat model of global brain ischemia/reperfusion injury. All single, double, triple, and quadruple wavelength combinations that reduce COX activity were evaluated for neuroprotection using a randomized and blinded study design (15 treatment groups). IRL was applied at the onset of reperfusion and maintained for 2 h. After 14 days, brains were analyzed via counting of CA1 hippocampal neurons. In untreated animals subjected to global brain ischemia 88% of neurons were lost. Strikingly, for the 15 IRL combination groups, loss of neurons ranged from only 11% for the best treatment regimen to 42%. The significant neurologic protection observed in IRL treated rats also coincided with preservation of neurologic function. To gain further insight into the mechanism of neuroprotection conferred by IRL we utilized *in situ* detection of mitochondrial superoxide generation. In untreated animals ischemia/reperfusion induced a 7-fold increase in MitoSOX fluorescence, indicative of mitochondrial ROS production, whereas animals treated with IRL showed fluorescent signals similar to controls. These data demonstrate the potential neuroprotective effect of non-invasive modulation of mitochondrial function with specific IRL.

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